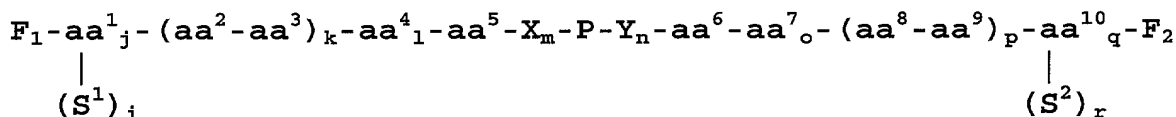


## CLAIMS

**WHAT IS CLAIMED IS:**

1. A fluorogenic composition for the detection of the activity of a protease, said composition having the formula:



10 wherein, P is a peptide selected from the group consisting of IETDSGV (SEQ ID NO: 208), SEVNLD AEF (SEQ ID NO: 209), and YVHDAPV (SEQ ID NO: 210);

$F^1$  and  $F^2$  are fluorophores and  $F^1$  is attached to the amino terminal amino acid and  $F^2$  is attached to the carboxyl terminal amino acid;

$S^1$  and  $S^2$ , when present, are peptide spacers ranging in length from 1 to about 50 amino acids and  $S^1$ , when present, is attached to the amino terminal amino acid and  $S^2$ , when present, is attached to the carboxyl terminal amino acid;

i, j, k, l, m, n, o, p, q, and r are independently 0 or 1;

$aa^1$  and  $aa^{10}$  are independently selected from the group consisting of lysine, ornithine and cysteine;

$aa^2$ ,  $aa^3$ ,  $aa^8$ , and  $aa^9$  are independently selected from the group consisting of an amino acid or a dipeptide consisting of Asp, Glu, Lys, Ornithine, Arg, Citulline, homocitrulline, Ser, homoserine, Thr, and Tyr;

$aa^5$ ,  $aa^4$ ,  $aa^6$ , and  $aa^7$  are independently selected from the group consisting of proline, 3,4-dehydropyrroline, hydroxyproline, alpha aminoisobutyric acid and N-methyl alanine;

X is selected from the group consisting of Gly,  $\beta$ Ala,  $\gamma$ Abu, Gly-Gly, Ahx,  $\beta$ Ala-Gly,  $\beta$ Ala- $\beta$ Ala,  $\gamma$ Abu-Gly,  $\beta$ Ala- $\gamma$ Abu, Gly-Gly-Gly,  $\gamma$ Abu- $\gamma$ Abu, Ahx-Gly,  $\beta$ Ala-Gly-Gly, Ahx- $\beta$ Ala,  $\beta$ Ala- $\beta$ Ala-Gly, Gly-Gly-Gly-Gly, Ahx- $\gamma$ Abu,  $\beta$ Ala- $\beta$ Ala- $\beta$ Ala,  $\gamma$ Abu- $\beta$ Ala-Gly,  $\gamma$ Abu- $\gamma$ Abu-Gly, Ahx-Ahx,  $\gamma$ Abu- $\gamma$ Abu- $\beta$ Ala, and Ahx-Ahx-Gly;

Y is selected from the group consisting of Gly,  $\beta$ Ala,  $\gamma$ Abu, Gly-Gly, Ahx, Gly- $\beta$ Ala,  $\beta$ Ala- $\beta$ Ala, Gly- $\gamma$ Abu,  $\gamma$ Abu- $\beta$ Ala, Gly-Gly-Gly,  $\gamma$ Abu- $\gamma$ Abu, Gly-Ahx, Gly-

Gly-βAla, βAla-Ahx, Gly-βAla-βAla, Gly-Gly-Gly-Gly (SEQ ID NO: 211), γAbu-Ahx, βAla-βAla-βAla, Gly-βAla-γAbu, Gly-γAbu-γAbu, Ahx-Ahx, βAla-γAbu-γAbu, and Gly-Ahx-Ahx; and

when i is 1, S<sup>1</sup> is joined to aa<sup>1</sup> by a peptide bond through a terminal alpha amino group of aa<sup>1</sup>; and when r is 1, S<sup>2</sup> is joined to aa<sup>10</sup> by a peptide bond through a terminal alpha carboxyl group of aa<sup>10</sup>.

2. The composition of claim 1, wherein the carboxyl terminal amino acid in which the carboxylic acid group is replaced with an amide.

3. The composition of claim 1, wherein:

r is zero; and

aa<sup>10</sup> has a C-terminal amide group or free carboxylic acid group.

4. The composition of claim 1, comprising an amino acid sequence selected from the group consisting of KDPJGYVHDAPVGJPKGY, KDPJGYVHDAPVPGKY, and KDPYVHDAPVGJPKGY.

5. The composition of claim 4, wherein said composition has a terminal blocking group.

6. The composition of claim 4, wherein said composition has a terminal Fa group.

7. The composition of claim 4, wherein said composition has a terminal Fmoc group.

8. The composition of claim 1, comprising the amino acid sequence - KDBJGSEVNLD A EFGJPKDDY.

9. The composition of claim 1, wherein F<sup>1</sup> and F<sup>2</sup> are the same fluorophore.

10. The composition of claim 9, wherein said F<sup>1</sup> and F<sup>2</sup> have an excitation wavelength between about 315 nm and about 800 nm.

11. The composition of claim 1, wherein the F<sup>1</sup> molecule is attached through either an  $\alpha$ -amino group of the aa<sup>1</sup> amino acid or through a side chain amino group of the aa<sup>1</sup> amino acid, or through a sulfhydryl group of a side chain of the aa<sup>1</sup> amino acid.

12. The composition of claim 1, wherein the F<sup>2</sup> molecule is attached either through a side chain amino group of the aa<sup>10</sup> amino acid, through a carboxyl group of the aa<sup>10</sup> amino acid, or through a sulfhydryl group of a side chain of the aa<sup>10</sup> amino acid.

13. The composition of claim 1, wherein said fluorophore is selected from the group consisting of rhodamine X, 9-(2,5 (or 2,6)-dicarboxyphenyl)-3,6-bis(dimethylamino)xanthyliumhalide or other anion (TMR), 9-(2,5 )-dicarboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylium halide or other anion (Rh6G), 9-(2,6 )-dicarboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylium halide or other anion, 9-(2,5 (or 2,6)-dicarboxyphenyl)-3,6-bisamino-xanthylium halide or other anion (Rh110), 9-(2,5 (or 2,6)-dicarboxyphenyl)-3-amino-6-hydroxy-xanthylium halide or other anion (Blue Rh), carboxytetramethylrhodamine, carboxyrhodamine-X , diethylaminocoumarin, 9-(2,5-dicarboxyphenyl)-3,6-bis-(dimethylamino)xanthylium chloride (5-TMR), 9-(2,6-dicarboxyphenyl)-3,6-bis-(dimethylamino)xanthylium chloride (6-TMR), 9-(2-carboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylium, 9-(2-carboxyphenyl)-3,6-bis(dimethylamino)xanthylium, and 9-(2-carboxyphenyl)-xanthylium.

14. The composition of claim 1, wherein said fluorophore comprises a carbocyanine dye.

15. The composition of claim 1, wherein said composition bears a hydrophobic group.

16. The composition of claim 1, wherein said composition bears a hydrophobic group.

17. The composition of claim 16, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorene-carboxylic group, 9-fluorene-carboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl

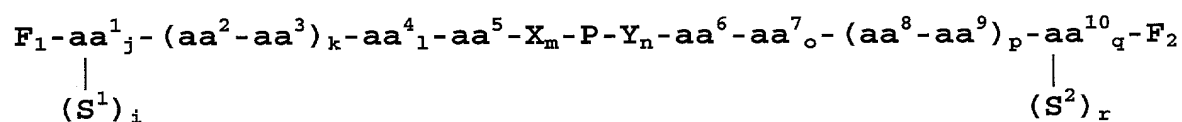
(Mbh), Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimethyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO), t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

18. The composition of claim 17, wherein said hydrophobic group is Fmoc.

19. The composition of claim 17, wherein said hydrophobic group is Fa.

20. The composition of claim 17, wherein said hydrophobic group is attached to the amino terminus of the molecule.

21. A fluorogenic composition for the detection of the activity of a protease, said composition having the formula:



wherein, P is a peptide selected from the group consisting YVHDAPV (SEQ ID NO: 212), and dYVHDAPV (SEQ ID NO: 213);

$F^1$  and  $F^2$  are fluorophores and  $F^1$  is attached to the amino terminal amino acid and  $F^2$  is attached to the carboxyl terminal amino acid;

$S^1$  and  $S^2$ , when present, are peptide spacers ranging in length from 1 to about 50 amino acids and  $S^1$ , when present, is attached to the amino terminal amino acid and  $S^2$ , when present, is attached to the carboxyl terminal amino acid;

i, j, k, l, m, n, o, p, q, and r are independently 0 or 1;

$aa^1$  and  $aa^{10}$  are independently selected from the group consisting of lysine, ornithine and cysteine;

aa<sup>2</sup>, aa<sup>3</sup>, aa<sup>8</sup>, and aa<sup>9</sup> are independently selected from the group consisting of an amino acid or a dipeptide consisting of Asp, Glu, Lys, Ornithine, Arg, Citulline, homocitrulline, Ser, homoserine, Thr, and Tyr;

aa<sup>5</sup>, aa<sup>4</sup>, aa<sup>6</sup>, and aa<sup>7</sup> are independently selected from the group consisting of proline, 3,4-dehydropyrroline, hydroxyproline, alpha aminoisobutyric acid and N-methyl alanine;

X is selected from the group consisting of Gly, βAla, γAbu, Gly-Gly, Ahx, βAla-Gly, βAla-βAla, γAbu-Gly, βAla-γAbu, Gly-Gly-Gly, γAbu-γAbu, Ahx-Gly, βAla-Gly-Gly, Ahx-βAla, βAla-βAla-Gly, Gly-Gly-Gly-Gly, Ahx-γAbu, βAla-βAla-βAla, γAbu-βAla-Gly, γAbu-γAbu-Gly, Ahx-Ahx, γAbu-γAbu-βAla, and Ahx-Ahx-Gly;

Y is selected from the group consisting of Gly, βAla, γAbu, Gly-Gly, Ahx, Gly-βAla, βAla-βAla, Gly-γAbu, γAbu-βAla, Gly-Gly-Gly, γAbu-γAbu, Gly-Ahx, Gly-Gly-βAla, βAla-Ahx, Gly-βAla-βAla, Gly-Gly-Gly-Gly (SEQ ID NO: 214), γAbu-Ahx, βAla-βAla-βAla, Gly-βAla-γAbu, Gly-γAbu-γAbu, Ahx-Ahx, βAla-γAbu-γAbu, and Gly-Ahx-Ahx; and

when i is 1, S<sup>1</sup> is joined to aa<sup>1</sup> by a peptide bond through a terminal alpha amino group of aa<sup>1</sup>; and when r is 1, S<sup>2</sup> is joined to aa<sup>10</sup> by a peptide bond through a terminal alpha carboxyl group of aa<sup>10</sup>.

terminal alpha carboxyl group of aa<sup>10</sup>.

22. The composition of claim 21, wherein the carboxyl terminal amino acid in which the carboxylic acid group is replaced with an amide.

23. The composition of claim 21, wherein:

r is zero; and

aa<sup>10</sup> has a C-terminal amide group or free carboxylic acid group.

24. The composition of claim 21, comprising an amino acid sequence selected from the group consisting of KDBYVHDAPVPGGY (SEQ ID NO: 215), KDBGYVHDAPVGPGGY (SEQ ID NO: 216), KDBJGYVHDAPVGJPGGY (SEQ ID NO: 217), and KDBJGdYVHDAPVGJPGGY (SEQ ID NO: 218).

25. The composition of claim 24, wherein said composition has a terminal blocking group.

26. The composition of claim 24, wherein said composition has a terminal Fa group.

27. The composition of claim 24, wherein said composition has a terminal Fmoc group.

28. The composition of claim 21, wherein  $F^1$  and  $F^2$  are the same fluorophore.

29. The composition of claim 28, wherein  $F^1$  and  $F^2$  have an excitation wavelength between about 315 nm and about 800 nm.

30. The composition of claim 21, wherein the  $F^1$  molecule is attached through either an  $\alpha$ -amino group of the  $aa^1$  amino acid or through a side chain amino group of the  $aa^1$  amino acid, or through a sulfhydryl group of a side chain of the  $aa^1$  amino acid.

31. The composition of claim 21, wherein the  $F^2$  molecule is attached either through a side chain amino group of the  $aa^{10}$  amino acid, through a carboxyl group of the  $aa^{10}$  amino acid, or through a sulfhydryl group of a side chain of the  $aa^{10}$  amino acid.

32. The composition of claim 21, wherein said fluorophore is selected from the group consisting of rhodamine X, 9-(2,5 (or 2,6)-dicarboxyphenyl)-3,6-bis(dimethylamino)xanthylumhalide or other anion (TMR), 9-(2,5 )-dicarboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylum halide or other anion (Rh6G), 9-(2,6 )-dicarboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylum halide or other anion, 9-(2,5 (or 2,6)-dicarboxyphenyl)-3,6-bisamino-xanthylum halide or other anion (Rh110), 9-(2,5 (or 2,6)-dicarboxyphenyl)-3-amino-6-hydroxy-xanthylum halide or other anion (Blue Rh), carboxytetramethylrhodamine, carboxyrhodamine-X , diethylaminocoumarin, 9-(2,5-dicarboxyphenyl)-3,6-bis-(dimethylamino)xanthylum chloride (5-TMR), 9-(2,6-dicarboxyphenyl)-3,6-bis-(dimethylamino)xanthylum chloride (6-TMR), 9-(2-carboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylum, 9-(2-carboxyphenyl)-3,6-bis(dimethylamino)xanthylum, and 9-(2-carboxyphenyl)-xanthylum.

33. The composition of claim 21, wherein said fluorophore comprises a carbocyanine dye.

34. The composition of claim 21, wherein said composition bears a hydrophobic group.

35. The composition of claim 34, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorene-carboxylic group, 9-fluorene-carboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh), Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimethyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO), t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

36. The composition of claim 21, wherein said hydrophobic group is Fmoc.

37. The composition of claim 21, wherein said hydrophobic group is Fa.

38. The composition of claim 21, wherein said hydrophobic group is attached to the amino terminus of the molecule.

39. A method of detecting the activity of a protease, said method comprising contacting said protease with a composition of claim 1 or claim 21.

40. The method of claim 39, wherein said contacting is in a histological section.

41. The method of claim 39, wherein said contacting is in a cell culture.

42. The method of claim 39, wherein said contacting is in a tissue section.

43. The method of claim 39, wherein said contacting is contacting a seeded or cultured adherent cell.

44. The method of claim 39, wherein said contacting is in a cell suspension derived from a biological sample selected from the group consisting of a tissue, blood, urine, saliva, lymph, biopsy.

45. The method of claim 39, wherein said detecting is by a method selected from the group consisting of fluorescence microscopy, fluorescence microplate reader, absorption microplate reader, flow cytometry, fluorometry, absorption spectroscopy, and confocal fluorescent microplate reader.

46. A method of delivering a molecule into a cell, said method comprising: providing a molecule according to claim 1 attached to a hydrophobic group or to at least one fused ring structure; and contacting said cell with said molecule whereby said molecule enters said cell.

47. The method of claim 46, wherein said hydrophobic group is selected from the group consisting of: selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluoreneacetyl group, 9-fluoreneacetyl group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4-dimethoxybenzhydryl (Mbh), Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzoyloxycarbonyl (2-Cl-Z), 2-bromobenzoyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO), t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

48. The method of claim 46, wherein, said fluorophores are selected from the group consisting of rhodamine X, 9-(2,5 (or 2,6)-dicarboxyphenyl)-3,6-bis(dimethylamino)xanthyliumhalide or other anion (TMR), 9-(2,5 )-dicarboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylium halide or other anion (Rh6G), 9-(2,6 )-



dicarboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylium halide or other anion, 9-(2,5 (or 2,6)-dicarboxyphenyl)-3,6-bisamino-xanthylium halide or other anion (Rh110), 9-(2,5 (or 2,6)-dicarboxyphenyl)-3-amino-6-hydroxy-xanthylium halide or other anion (Blue Rh), carboxytetramethylrhodamine, carboxyrhodamine-X, diethylaminocoumarin, 9-(2,5-  
5 dicarboxyphenyl)-3,6-bis-(dimethylamino)xanthylium chloride (5-TMR), 9-(2,6-dicarboxyphenyl)-3,6-bis-(dimethylamino)xanthylium chloride (6-TMR), 9-(2-carboxyphenyl)-2,7-dimethyl-3,6-bis(ethylamino)xanthylium, 9-(2-carboxyphenyl)-3,6-bis(dimethylamino)xanthylium, and 9-(2-carboxyphenyl)-xanthylium.

49. The method of claim 46, wherein, said fluorophores are selected from  
10 the group consisting of: of carboxytetramethylrhodamine, carboxyrhodamine-X, diethylaminocoumarin, rhodamine 110, and a carbocyanine dye.

50. The method of claim 46, wherein, said cell is a mammalian cell.

51. A method of screening a test agent for the ability to modulate the activity of a protease, said method comprising:

15 contacting a protease or a cell comprising a protease with said test agent;

contacting said protease with a fluorogenic composition of any of claims 1 through 38; and

20 detecting a signal or lack of signal produced by said fluorogenic composition where a difference in the signal produced by the protease or cell contacted with said test agent compared to a control in which the protease or cell is contacted by said test agent at a lower concentration indicates that said test agent modulates activity of said protease.

52. The method of claim 51, wherein said test agent at a lower  
25 concentration is the absence of said test agent.

53. The method of claim 51, wherein an increase in signal produced by the protease or cell contacted with the test agent as compared to the control indicates that said test agent increases the activity of said protease.

54. The method of claim 51, wherein a decrease in signal produced by the protease or cell contacted with the test agent as compared to the control indicates that said test agent decreases the activity of said protease.

5 55. The method of claim 51, wherein said protease is contacted with the fluorogenic composition in the presence of the test agent.

56. The method of claim 51, wherein said protease is contacted with the fluorogenic composition after removal of the test agent.

10 57. The method of claim 51, further comprising entering test agents that modulate activity of said protease into a database comprising a list of test agents modulating said protease.

58. The method of claim 51, wherein said detecting comprises detecting an intracellular signal.

59. The method of claim 51, wherein said detecting comprises microscopy.

15 60. The method of claim 51, wherein said detecting comprises flow cytometry.

61. The method of claim 51, wherein said detecting comprises high-throughput screening of whole cells.